

Sudipto Das¹, Yoonsoo Hahn¹, Tapan Bera¹, Satoshi Nagata¹, Mark C. Willingham², Byungkook Lee¹ and Ira Pastan¹

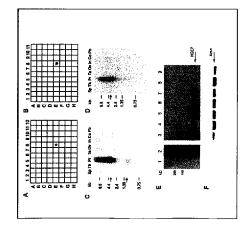


Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA and 2 Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, NC-27157, USA.

prostate cancer. RNA analysis revealed 2 splice variants of NGEP. A short form (NGEP-S) encodes a 20 Mac dysosing protein and a long form (NGEP-L) encodes a 96 Mac polybopic membrane protein. To study the location and the function of NGEP-L, we have generated a panel of monoclonal and polycional antibodies to the different regions of the NGEP-L. The antibodies detect a -100 kpc protein in extracts of normal prostate and prostate cancers that contains high levels of NGEP mRNA, immunohistochemistry of the prostate itsues using ambodies against NGEP-L shows that NGEP-L is shiply expressed on the apical and the lateral surfaces of normal prostate and prostate cancer cells. Lateral expression suggests that NGEP-L may have a role in prostate call interactions or adhesion. To study the fundion of NGEP-L may have a role in prostate call interactions or adhesion. To study the fundion of NGEP-L may expressing NGEP-L increase in density, the cells form aggregates. A specific RNAi that lowered NGEP-L leave towered tomation or of the cell aggregates. Our essits suggest that NGEP-L has a cone in promoting cell:cell contact dependent interactions of prostate cells. Asto, because of its high expression in prostate carcer, its specific expression in prostate and its presence on the cell surface, NGEP-L is a promising stable LNCaP cell lines expressing NGEP-L at levels similar to those found in prostate tassues were generated. Confocal immuno-fluorescence studies of these cell lines showed that NGEP-L is present in the plasma-membrane and is highly concentrated at the cell:cell contact regions. Furthermore, as the cells expression of the NGEP (TMEM16G) gene has only been detected in normal arget for the antibody-based therapies of prostate cancer

**EXHIBIT D** 

# Tissue-specific expression of NGEP



# Fig. 1. Tissue-specific expression of NGEP mRNA and protein

	£												_				
									_								
€€	€																
€€	€	2.2	E J	E T	EE.	ES	D.T.	D.T.	Œ	Œ	Œ	CI.	EI	LI	EE	Œ	Œ
nemal cett. 1 Dang cuncer with serval cett. 2	Perma cause unit	17 (484) P	trans.3	7 900	3.3	Frightwok -7 frightwot -2	Personal Processor	2000	School Sound School Subse	Selver 2 c Service	Machine man in it.	Amade de Good II. d. Semanda de Good II. d.	4644 7 5,844 .	Months J. J.	A shared A	Section states 2	5,000
	€€	€€€	€ € € ≥3	€ € € 2222	€ € € 2222 25	€ € € 2202 22 22	€ € € 2322 25 22 22	€ € € 5300 55 53 53 53	€ € € 22°22 22 22 22 22 22	€ € € 2702 22 22 22 22 22 22	€ € € 2222 25 25 25 25 25 25 25 23	€ € € 23°22 25 25 25 25 25 25 25 25	€ € € 53000 25 20 20 20 20 20 20 20 20 20 20 20 20 20	€ € € 5300 55 55 55 53 53 53 53 53 53 53 53	€ € € 2222 23 23 23 23 23 23 23 23 23 23 23 2	€ € € 2222 25 25 25 25 25 25 25 25 25 25 25 2	€ € € 2222 23 23 23 23 23 23 23 23 23 23 23 2

#### (\*) Politics chaining with LSC: (\*) No Phining with LSC: Wetweethand

## Immunohistochemical analysis of NGEP-L with Mab 19C3.

Fig. 2. NGEP-L is present in the cell:cell contact regions in the LNCaP-NGEP cells

	_		_		_		_	т																										
												_	_		_							_	_			_		_	_			,	,	
	4	Ē		€		÷							•																					
	÷	Ξ		 €		ŧ					ī		Ē:	ī	:	Ξ	EI		II	ī	Ţ.	33	-	Œ	Œ	0	I	IJ	,	LI	ΙΙ	Ξ	τī	
CHOCKER CONCRETED	nemologie.		1	 STREET, SEE. 2	The same content	Commercial Trailing		17.1437.04	200	10000			1	1 500	2.729	3	go spengilong go spengilong		Contract of	106,813	*.	Agree Agree		1.04	Machine mach 2	The section of the se	South Bush J	1 2196		Manual J	Abries 7 Abries 2	A section and	2 0 mg/s	
		_	_	 				-							_			_		-			+				٠.							

### Fig. 3. Location of NGEP-L in prostate.

Silisto were propued from formalls frost, paraffic-ambedded human prosible center samples and NGEP. production of the propulation of the production of the

#### Expression of NGEP-L causes aggregation of LnCAP cells.

NGEP is localized in cell-cell contact regions

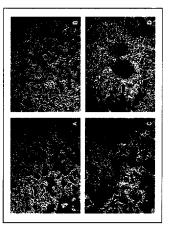
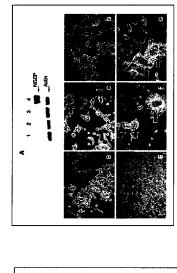


Fig. 4. Expression of NGEP-L in the LNCaP cells causes the aggregation of the cells.

Phrose-contrast photographs of growing LNCaP cells. A, parental LNCaP cells. B, LNCaP-Vectos cells. C, LNCaP-C, C, LNCaP-C, C, LNCaP-M, CAPP. MCGP. M cells. C, LNCaP-More more cells c, LNCaP-M, cells and the cells of cells. A trows, a gargagican of the cells due to NGEP. morphology compared with the centrel cells. A trows, a gargagican of the cells due to NGEP.

his attitude of NGEP-L was done that attitude of NGEP-L was done that attitude of Company of Armwak NGEP-L was animal with goldin-8 anihody wg. Armwhead, colocalization (yellow) of all yellow of the Company of the Co



# Fig. 6. NGEP-L siRNA mediated gene silencing in LNCaP-NGEP cells reverses the aggregation phenotype.

1. Benr TK, Dos S, Maede H, Beens R, Wolfgang CD, Kumar V, Hahn Y, Lee B, Padan II, Phoc Nati Acad Sci U S A. 2004.(1015)905944. 2. Dos S, Hain Y, Nagana S, Willingham MC, Ben TK, Lee B, Pastan II, Cancar Res. 2007.Fish 15-87(4):1594-501.